a more general platform for the evolution of novel enzyme activities. (This work was supported by NSF Grant No. MCB-0615938 to R. M. W. and NIH Grant No. U54 GM094599 to R. M. W. and S. B. O.).

1911-Pos Board B48
A Multi-Pronged Approach for Uncovering Allosteric Networks in Caspases
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Caspases, the cysteine proteases that initiate and control apoptotic cell death, are subject to allosteric regulation by a variety of modulators. Due to their role in cell death, caspases are of interest as drug targets for diseases ranging from cancer to neurodegeneration. The most significant hurdle to their therapeutic use appears to be related to the overlapping active-site specificities for small molecule inhibitors, which do not fully reflect their true in vivo specificities for protein substrates. Due to this complication, allosteric inhibition of individual caspases or particular caspase sub-functions is of great interest. Fortunately, caspases are extremely amenable to allosteric regulation, in large part due to their remarkably plastic substrate-binding grooves, which can be modulated by a number of distinct allosteric mechanisms. Our current work is to develop a global map of the allosteric networks across the family. We have discovered allosteric sites in caspase-6 and -9 that are natively regulated by zinc and elucidated the molecular mechanism of inhibition crystallographically. We have identified other allosteric sites, unique to caspase-3, -6, -7, -8 or -9 respectively, which are controlled by phosphorylation. The allosteric networks including these sites utilize distal control of the substrate-binding groove. Based on our understanding of these mechanisms of inhibition, we have engineered an allosterically handcuffed version of caspase-7 that can be unlocked by the intracellular reduction potential. Finally, using specially-designed nanoparticles we have delivered caspases and induced apoptosis in cancer cells. Together these findings move us closer to a full understanding of the allosteric networks controlling caspase function and therapeutically relevant allosteric control of caspases.

1912-Pos Board B49
Regulation of Kinases: 1 Billion Years of Evolution
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Protein phosphorylation is an essential regulatory mechanism that affects all aspect of cellular life from division and growth to aging and death. Misregulation of the signaling cascades leads to severe detrimental effects, and in humans often associated with cancer and other diseases. Phosphorylation is performed by a class of protein called kinases. Activation and deactivation of kinases is normally under tight control and is regulated via different mechanisms that are incredibly complex. In this work we combine phylogenetic resurrection techniques with biophysical and chemical approaches to analyze the regulatory mechanisms of modern tyrosine oncokinases Src and Abl, their common ancestor and the common ancestors between several other families of tyrosin kinases. Our results show how the regulatory elements appeared and developed throughout the evolution enabling selective regulation of complex modern cascades.

Membrane Protein Interactions

1913-Pos Board B50
Interactions of Dok7 with Model Membranes Containing Anionic Lipids and Phosophoinositides
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The receptor tyrosine kinases (RTKs) are a major class of transmembrane receptors responsible for regulation of many biological processes, including development and maintenance of synapses. Many RTKs act in concert with intracellular proteins which are thought to interact with cell membranes. Downstream-of-Kinase 7 (Dok7) is a soluble protein involved in the Muscle-Specific Kinase (MuSK) signalling pathway. Dok7 binds to MuSK to facilitate clustering of acetylcholine receptors at synapses. Mutations in Dok7 have been known to result in varying degrees of severity of congential myasthenic syndromes, which is characterised by impaired muscle contractions. The structure of Dok7 reveals it to contain a pleckstrin-homology (PH) and a phosphotyrosine-binding (PTB) domain. Although there is evidence to suggest how Dok7 interacts with MuSK, it is unknown exactly how it interacts with the cell membrane.
Here we apply a multi-scale Molecular Dynamics simulations method to characterise Dok7's interactions with membranes of varying lipid composition. Coarse-grained (CG) simulations are used to characterise the mechanism of which Dok7 binds to the bilayer, while atomistic simulations are used to refine its interactions with specific lipids present in the bilayer. These computational studies reveal the role of phosphatidylinositol phosphates (PIP) in the interaction of Dok7 with complex cell membranes.

1914-Pos Board B51
The Difference in Arl2 and Arl3 Membrane Binding and Localization
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ADP-ribosylation factor-like (Arl) proteins are small GTPases, with Arl2 and Arl3 being close homologues that share almost all their interacting partners. Despite all similarities, Arl2 and Arl3 have distinct biological functions: Arl3 is regarded as a ciliary protein, whereas Arl2 has been reported to be involved in tubulin folding and Ras signaling. Defective ciliary function results in a number of human diseases. So far, how are these different roles attained by the two homologue proteins is not a fully answered question.
A recent study showed that the N-terminal amphipathic helix of Arl3 but not Arl2 can function as a GTP-dependent pocket opener, displacing myristoylated cargo from the lipid-binding pocket of the GDI-like solubilizing factor UNC119a/b [1]. This would imply that membrane-bound Arl3•GTP is not able to bind UNC119a/b, since this helix is predicted to mediate Arl3 membrane binding and is only exposed in Arl3•GTP, thus connecting the membrane binding capacity of Arl to its nucleotide status and the availability of the N-terminal helix.
In the present study, the membrane binding behavior of Arl3, Arl2, and UNC119a has been investigated by surface plasmon resonance, atomic force microscopy, and infrared reflection absorption spectroscopy to gain insight into the role of the N-terminal amphipathic helix of Arl2/3 during membrane binding and its modulation by complexation with UNC119a. The data reveal a preferential localization of Arl2/3 in the liquid-disordered phase of heterogeneous model membranes. Unlike Arl3 and other Arf proteins, Arl2 binds to membranes in a nucleotide-independent manner. Finally, UNC119a selectively impedes membrane binding of Arl3•GTP.
Reference
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1915-Pos Board B52
Regulation of K-Ras Membrane Association: Calmodulin Versus PDEδ
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Ras is a small GTP-binding protein and involved in a variety of cellular processes. The isoform K-Ras4B binds with its polybasic farnesylated C-terminus to membranes and can enter multiple interactions with a wide variety of effectors. PDEδ and calmodulin (CaM) are known to function as potential binding partners for farnesylated Ras proteins, leading to a modulation of the dynamics of Ras membrane association. A previous study of our group showed that PDEδ is not able to extract K-Ras4B from model raft membranes; instead, an effective delivery of PDEδ-solubilized K-Ras4B to the plasma membrane was proposed [1]. Since CaM exhibits additional interaction sites to the G-domain of K-Ras4B as compared to PDEδ and was shown not to be required for the transport of K-Ras4B to the plasma membrane, it was suggested that calmodulin dissociates K-Ras4B from membranes [2]. In the present biophysical approach, the influence of CaM on the interaction of K-Ras4B with anionic model raft membranes has been investigated by surface plasmon resonance, atomic force microscopy and fluorescence anisotropy measurements, supplemented by infrared reflection absorption spectroscopy experiments. The results suggest a repulsion of the K-Ras4B/CaM complex from the membrane. At the end, differentiation between the function of the two farnesyl-binding proteins on K-Ras4B is envisaged.
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